(ii) adjusting the pH to an acid pH with a strong acid;

(iii) separating the mixture from step (ii) into two layers;

(iv) removing the upper layer and adjusting its pH to approximate neutrality;

(v) adding to the product from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi) adjusting the pH of the product from step (v) to an alkaline pH with a weakly alkaline aqueous solution; and (vii) separating out the cell wall C-polysaccharide antigen containing not more than 10% protein;

C' cont

c) coupling to a chromatographic column through a spacer molecule the cell wall C-polysaccharide antigen containing not more than 10% protein obtained in step (b);

d) passing polyvalent antibodies to *Streptococcus pneumoniae* over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and

e) conducting an immuno-assay upon a liquid sample suspected of containing *Streptococcus pneumoniae* and/or its C-polysaccharide cell wall antigen which immuno-assay comprises the steps of

(i) contacting the liquid sample with conjugates of purified antigen specific antibodies from step(d) hereof and a labelling agent capable of manifesting a color or a detectable signal upon completion of the immunoassay, whereupon C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in the sample,

whether or not in free form, will react with said conjugates to form labelled antibody-antigen conjugates,

(ii) further contacting the liquid and all of the conjugates it contains with a solid surface upon which a mass of unlabelled antigen-specific antibodies from step (d) hereof have been immobilized, whereupon any labelled antibody-antigen conjugates present will react with the immobilized antibodies on the surface to form labelled antibody-antigen-immobilized antibody sandwiches, and

C' cont

(iii) detecting any label thereby accumulated on the solid surface by a detection means appropriate to the nature of the label so as to confirm the presence of the *Streptococcus pneumoniae* C-polysaccharide cell wall antigen in the sample.

34 The method of claim 33 in which the spacer molecule of step (c) is a protein molecule.

35 The method of claim 33 wherein the sample of step (e) is a natural liquid of mammalian origin.

36 The method of claim 35 wherein the liquid sample of step (e) is human urine.

37 The method of claim 36 in which the liquid sample is taken from a patient exhibiting clinical signs of pneumonia.

38 The method of claim 36 in which the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

39 The method of claim 35 wherein the liquid sample of step (e) is human spinal fluid.

40 The method of claim 39 wherein the sample is obtained from a patient suspected of having meningitis.

(41-Cancelled)

42 The method of claim 33 in which step (e) is an immunochromatographic ("ICT") process.

43 The method of claim 42 in which step (e) is conducted by

a) contacting a liquid sample suspected of containing *Streptococcus pneumoniae* and/or its free cell wall C-polysaccharide antigen, with the sample-receiving end of a strip of bibulous material, which strip is contained within an ICT device comprising a housing and itself comprises

(i) a first zone in which has been movably embedded a conjugate of a labelling agent with purified antigen-specific antibodies obtained in step (d) of claim 33, said labelling agent being selected from among those known to manifest a visible color change upon the formation of a labelled antibody - antigen - fixed antibody reaction product and

(ii) a second zone having fixedly bound thereto a stripe of unconjugated purified antigen-specific antibodies from step (d) of claim 33, which zone is equipped with a window in the housing for viewing the appearance of a color characteristic of the massing of the labelling agent upon the formation of the labelled antibody - antigen - fixed antibody reaction product;

b) allowing said liquid sample to flow laterally along said test strip to said first zone where it picks up the movably embedded conjugate of labelling

agent and antigen-specific antibodies obtained in step(d) of Claim 33

c) allowing said liquid sample and said conjugate of antigen-specific antibodies to flow laterally together along said test strip to said second zone while concomitantly reacting to form labelled antibody-antigen conjugates with C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*, free or combined, present in the sample and

d) within not more than 20 minutes after first contacting the liquid sample with the test strip, observing, through said window in the housing whether a line of color has formed, indicative of the massing of said label along the stripe of unconjugated purified antibodies, as labelled antibody-antigen-fixed antibody reaction products are formed.

44 The method of claim 43 wherein the sample is a natural liquid of mammalian origin.

45 The method of claim 44 wherein the sample is human urine.

46 The method of claim 45 wherein the sample is taken from a patient exhibiting overt clinical signs of pneumonia or another respiratory tract illness known to be often caused by *Streptococcus pneumoniae*.

47 The method of claim 44 wherein the liquid sample is human spinal fluid.

48 The method of claim 45 wherein the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

49 The method of claim 45 wherein the liquid sample is taken from a patient suspected of having meningitis.

50 An ICT device for the detection of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in a liquid sample, which device comprises a housing containing a strip of bibulous material having

a) a first zone in which has been movably embedded a conjugate of a labeling agent and purified antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, and

b) a second zone downstream of said first zone having immovably bound thereto a stripe of purified antibodies specific to the same cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, which zone is equipped with a window in the housing for viewing the appearance of a line of color along said stripe, which color is indicative of the massing of the labelling agent along the immovably bound stripe as a consequence of the formation of labelled antibody-antigen-immovable antibody sandwiches, whereby the line of color denotes the presence in the liquid sample of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*;

all of which antibodies in both zones are further characterized in that their antigen specificity has been attained by passing polyvalent antibodies to *Streptococcus pneumoniae* over a chromatographic affinity column to which is coupled a spacer molecule conjugated to a purified cell wall C-polysaccharide antigen obtained from a culture of *Streptococcus pneumoniae* bacteria according to the following method:

(i) harvesting cells from the said culture in the form of a wet cell pellet;

(ii) suspending the wet cell pellet in an alkaline solution and mixing;

(iii) adjusting the pH of the resultant mixture to an acid pH with a strong acid;

(iv) separating the acidified product from step (iii) into two layers;

(v) removing the upper layer and adjusting its pH to approximate neutrality;

(vi) adding to the product from step (v) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vii) adjusting the pH of the product from step (vi) to an alkaline pH with a weakly alkaline aqueous solution; and

(viii) separating out the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* having no more than 10% protein.

51 The ICT device of claim 50 wherein the labelling agent is finely divided metallic gold.

52 A method for detecting the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* in a liquid sample which comprises

a) contacting said sample with the sample receiving end of the strip of bibulous material contained in the ICT device of claim 50;

b) allowing the liquid sample to flow laterally to the first zone of said test strip where it picks up the movably embedded conjugates of labelling agent and purified antigen -specific antibodies;

c) allowing the liquid sample and the said conjugates to flow laterally

together along said test strip to the second zone thereof, while the conjugates concomitantly react with cell wall C-polysaccharide antigen of *S. pneumoniae* if present in the sample, whether free or bound, to form labelled antibody-antigen conjugates, and

d) within approximately 15 to 20 minutes after the initial contact of the sample with the test strip, observing through the view window whether a line of color has appeared along the stripe of immovably bound antibodies, which line of color indicates massing of label along the stripe due to reaction of the labelled antibody- antigen conjugates formed in step(c) with the immovably bound antibodies to form "sandwiches", thereby indicating the presence in the liquid sample of the cell-wall C-polysaccharide antigen of *Streptococcus pneumoniae*.

53 The method of claim 52 wherein the liquid sample is of natural mammalian origin.

54 The method of claim 53 wherein the liquid sample is selected from among human urine, human sputum and human spinal fluid.

REMARKS

I. General Statement

The claims in this application are 1-9, 33-40, and 42-54. Claims 1-9 are retained herein pending the filing of a divisional application.

By this amendment, Claim 41 has been canceled and the remaining claims have been extensively rewritten in an effort to meet the various claim rejections made in the most recent